Lipid-modifying enzymes play a vital role in the regulation of lipids as mediators of cell function. One example is the hydrolysis of phospholipids through phospholipase D (PLD), which produces the signalling molecule phosphatidic acid (PA). These processes at lipid membranes can be observed in situ through the application of different biophysical techniques. Thus, the hydrolysis of phosphatidylcholines by PLD was investigated, showing that the enzyme is highly affected in its catalytic activity by the lipid membrane structure. Briefly, by using Langmuir monolayers as a model system, we revealed that PLD activity depends on the segregation of the hydrolysis product PA within the monolayer. Hence, we could describe how the structure of the PA-rich domains is decisive for the activation and inhibition of PLD. This study demonstrates how membrane structure influences the activity of PLD and regulates the concentration of the lipid messenger PA.
The current research project is aiming at describing a toxic component of the venom of brown spiders (Loxosceles), which has a rare enzymatic activity termed sphingomyelinase D (SMD). SMD catalyzes the conversion of sphingomyelin (SM) into ceramide-1-phosphate (Cer-1-P). While the enzymatic substrate SM is an integral constituent of many cell membranes, especially in the vascular epithelium and red blood cells, the reaction product Cer-1-P occurs in very low concentrations. Cer-1-P is suggested to be a novel lipid second messenger in cellular signal transduction events. At present, the precise mechanism of venom action is incompletely understood, but preliminary results show the strong effect of SMD activity on the membrane structure of giant unilamellar vesicles. In summary, the presented work depicts the correlation between membrane structures and the activity of lipid-modifying enzymes. This implements new models for the regulation of cellular processes through distinct structures of biological membranes.

1948-Plat
Action Of The Antimicrobial Peptide Novicidin: Divorcing Folding From Function
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Many small cationic peptides have antimicrobial properties. This is assumed to be linked to their ability to permeabilize bacterial membrane. Membrane binding is usually accompanied by the transition from an unstructured conformation to an α-helical state. To investigate further the link between folding and membrane permeabilization we have studied the effect of acylating the N-terminus of the antimicrobial peptide Novicidin with C8, C12 and C16 chains. Acylation increases the ability to form α-helical structure in the presence of zwitterionic vesicles but *reduces* the ability to permeabilize these vesicles, even at concentrations sufficiently low to prevent formation of peptide micelles mediated by the acyl chains. Laser confocal scanning microscopy studies that show Novicidin's preference for DOPC vesicles among populations of different vesicles. The divorce between folding and function is further emphasized by stopped-flow studies using fluorophor-labelled peptide which indicate that a more superficial mode of binding is more efficient in releasing vesicle contents. Rapid kinetic measurements showed a significant increase in the vesicle disruption lag time as a function of acyl chain length indicating that acylation actually decreases the kinetics of interaction. We suggest that induction of α-helical structure is not a prerequisite for membrane disruption but may in fact inhibit disruption by sequestering the peptide in less membrane-active conformations inserted deeper into the membrane than the non-acylated form. This is corroborated by surface-measurements using Quartz Crystal Microbalances with Dissipation and Dual Polarization Interferometry. Our microscopy studies also reveal multiple modes of interaction between AMPs and simple model membranes, namely fusion, pore-formation and lysis, and indicate that peptide-membrane interactions may be even more varied in the complex environment of live bacterial membranes.

1949-Plat
Membrane Tubulation by Lattices of Amphiphysin BAR Domains
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Membrane compartments of manifold shapes are found in cells, often sculpted by cellular proteins. In particular, proteins of the BAR domain superfamily participate in membrane sculpting processes in vivo and reshape also in vitro low-curvature membrane liposomes into high-curvature tubes and vesicles, achieving their role by binding with their curved, positively charged surfaces to negatively charged membranes. Recent observations revealed that membranes are shaped actually through the concerted action of multiple BAR domains arranged in a lattice. However, information on the dynamics of membrane bending and an explanation of the lattice's role are still lacking. Here we show by means of coarse-grained molecular dynamics simulations totaling over 1 millisecond, how lattices involving parallel rows of amphiphysin BAR domains sculpt flat membranes into tubes. A highly detailed, dynamic picture of the formation of membrane tubes by lattices of BAR domains over time scales of 100 microseconds is obtained. Lattice types inducing a wide range of membrane curvatures are explored. The results suggest that multiple lattice types are viable for efficient membrane bending. The lattices found to be optimal for producing high membrane curvature are composed of protein rows separated by 5 nm, stability of the rows being maintained through electrostatic interactions between BAR domains.

1950-Plat
Probing the Interaction of Charged Lipids with the Potassium Channel KcsA
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The activity of integral membrane proteins has long been known to be tightly coupled to the lipid composition of the surrounding lipid bilayer. More recently though the presence of non-annular lipid binding sites have been shown to play a key role in the regulation of membrane channels. In particular recent fluorescence studies have revealed that gating of the potassium channel KcsA is highly dependent on the binding of anionic lipids to three or more non-annular lipid binding sites at the lipid protein interface[1]. Here we present solid-state NMR studies on KcsA reconstituted into charged lipid bilayers composed of POPC/POPG. These studies are allowing us to investigate the nature of the interaction between the surrounding lipid and these binding sites.
Employing ^{1}H-^{31}P saturation transfer MAS NMR[2] we have been able to probe the proximity and rate of exchange of lipid in close proximity to the KcsA. A significant attenuation of the POPC resonance was observed upon the saturation of amide protons suggesting that POPC populates the annular sites of KcsA and is in relatively fast exchange with the bulk lipid. In contrast no such attenuation was observed for the POPG, which in light of earlier fluorescence studies suggests that the POPG remains resident at the lipid protein interface and does not readily exchange with the bulk lipid. Preliminary heteronuclear correlation spectra in conjunction with T_2 filtering are beginning to provide us with insights into the types of residues involved in this interaction.
1) P. Marius *et al.*, Binding of anionic lipids to at least three nonannular sites on the potassium channel KcsA is required for channel opening. *Biophysical Journal* 2008 (94)1689-98.
2) *O. Soubias et al.*, Evidence for lipid specificity in lipid-rhodopsin interactions Journal of Biological Chemistry 2006 (281)33233-41.

1951-Plat
Folding and Assembly of Membrane Proteins: Coarse Grained Molecular Dynamics Simulations of EmrE
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EmrE is a bacterial drug resistance transporter, from E. coli. It is believed to function as an antiparallel homodimer, each monomer of which contains four transmembrane helices. Coarse-grained molecular dynamics (CG-MD) simulations have been previously used to study the insertion and self-assembly of transmembrane helices, and the formation of transmembrane helix dimers and tetramers in lipid bilayers. Such simulations have used a local modification of the original Marrink CG forcefield [1]. In the current study, these methods are employed to investigate the folding and self-assembly of EmrE. Self-assembly CG-MD simulations of the isolated helices of EmrE suggest that each of the constituent helices inserts into a phosphatidylcholine bilayer to adopt a transmembrane orientation. Helix hairpins and other fragments have been simulated to explore the self-assembly and folding processes of the protein subsequent to helix insertion. Simulations of parallel vs. anti-parallel pairs of EmrE monomers are used to explore formation and stability of the EmrE dimer.
(1) Bond, P.J., Wee, C.L., and Sansom, M.S.P. (2008) Coarse-grained molecular dynamics simulations of the energetics of helix insertion into a lipid bilayer. Biochem. (in press), bi-2008-00642m.R1.

1952-Plat
Molecular Dynamics Simulations of Apolipoprotein A-I Peptide Mimetic 4F
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Coronary heart disease is the leading cause of death in the United States, claiming more lives than the next seven leading causes of death combined. High levels of high density lipoprotein (HDL) have been correlated with lower rates of coronary heart disease. Apolipoprotein A-I (apoA-I), is the principle protein in HDL, is a 243-residue class A amphipathic alpha helix capable of binding a variable number of lipid molecules. ApoA-I mimetic peptides synthesized by Anantharamaiah et al. are 18-residue class A amphipathic helices. Although